



Research Article

ANTI-ULCER EFFECT OF *NIGELLA SATIVA* LINN. AGAINST GASTRIC ULCERS IN RATS

Mohd Wasif Ahmed*, Roshan.S, N.L. Mahammed, M A Muqeem Nasir

Department of Pharmacology, AZAD College of Pharmacy, Moinabad, Telangana, India.

*Corresponding author's Email: mohammad.pharma2014@gmail.com

(Received: November 22, 2015; Accepted: January 08, 2016)

ABSTRACT

The effect of alcoholic extract of *Nigella Sativa* was investigated in rats to evaluate the anti-ulcer activity by using two models, i.e. Pyloric ligation and aspirin Induced gastric ulcer. The parameters taken to assess anti ulcer activity were volume of gastric secretion, free acidity, total acidity and ulcer index. The results indicate that the alcoholic extract significantly ($P < 0.001$) decreases the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control.

Keywords: *Nigella Sativa*, Pyloric Ligation, Aspirin, Ulcer Index.

INTRODUCTION

Peptic ulcer is one of the major gastrointestinal disorders, which occur due to the imbalance between gastric aggressive and defensive factors¹. Various other factors which contribute for the formation of gastric ulcers are *Helicobacter pylori* infection, frequent use of NSAIDs, consumption of alcohol & nicotine etc². Anti-ulcer drugs used in the treatment of gastric ulcers are reported to show various side effects like nausea, constipation, abdominal pain and diarrhea. Ulceration is reported for high chances of recurrence and mortality. Thus there is a need for more effective and safe anti-ulcer agents aiming to relieve pain, heal the ulcer and delay ulcer recurrence. Therefore herbal medicines are considered safer alternatives because of natural ingredients with no side effects³. However, plant extracts are the most important sources of herbal medicine and new drug development which produce efficient results in treatment of gastric ulcers. In countries like India, flowers are always used in all its cultural rituals. Flowers are widely used for their beauty and the

color they radiate. Flowers which serve their purpose usually wilt and are thrown a trash.

Nigella sativa Linn., a plant belonging to the family Ranunculaceae, grows as a small herb and is cultivated throughout India and other tropical regions of the world. It is active as an aromatic, respiratory stimulant, diuretic, hypoglycemic, anti tumour and an analgesic.

The seed contains alkaloids nigellicin, nigellidin, quanazoline, tannin, steroid -spinasterol, campsterol, cholesterol, stigmas 7-en-3-beta-ol, stigmasterol and flavonoids of trigillin quercetin-3-glucoside. The study assumes significance in the context that prolonged use of synthetic anti-ulcer drugs leads to adverse drug reactions and a search for new anti-ulcer agents that retain therapeutic efficacy and are devoid of adverse drug reaction is warranted. A study of the efficacy of an extract of *N. sativa* in gastric ulcer with pylorus ligation and aspirin-induced ulcers was undertaken in a rat model.

MATERIALS AND METHODS

Plant Material: The fresh seeds of *nigella sativa* were collected from in and around Moinabad, telangana, India. Healthy seeds were chosen from the collected seeds and are dried in shade.

Extraction Process: The dried plant material was extracted in its entire form by Soxhalation, Maceration & Percolation.

1. Soxhalation: Dried plant material was extracted in soxhlet apparatus for 24hrs using methanol as a solvent.
2. Maceration: Dried plant material was macerated for 24 hours using water as menstrum.
3. Percolation: Dried plant material was extracted using methanol & water as solvents in 3:1 ratio for 7 days.
4. The seeds were dried and crushed into coarse powder which was used for extraction with alcohol (95% v/v) using Soxhlet apparatus. The extract was evaporated under vacuum. The extractive value (%w/w) of the alcoholic dry extract was 4.25%.

Animals: Wistar albino rats (140-200g) were taken to assess Anti-ulcer activity. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of Pharmacy College, (No.1516/PO/a/11/CPCSEA).

EVALUATION METHODS:

Pyloric Ligation Ulcer Model : Male albino rats weighing between 140 and 175 g were selected for pyloric ligation ulcer model⁴. Rats were divided into three groups, each group consisting of six animals. Animals were fasted for 24 h. One group received normal saline 2 ml/kg (negative control), the second group received ranitidine 20 mg/kg by oral route (positive control) and the third group received alcoholic extract of *N. Sativa* (150 mg/kg) by oral route, 30 min prior to pyloric ligation animals were sacrificed 4h later and the stomach was opened to collect the gastric contents. The total of gastric content was measured. The gastric were centrifuged at 1000 rpm for 10 min. one ml of the superatent liquid was pipeted out and diluted to 10ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity.

Aspirin-Induced Ulcer Model : In the aspirin-induced ulcer experiments⁵, three groups of albino rats (150–175 g), with each group consisting of six animals were used. The first group served as a control group, the second group served as positive control and the third group served as the test group. The second and third groups were treated respectively with ranitidine (20 mg/kg) and alcoholic extract of seed of *N. sativa* (150 mg/kg), orally for 8 days. Control animals received normal saline (2ml/kg) for 8 days. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (a dose of 200 mg/kg orally) on the day of sacrifice. The animals were sacrificed 4 h later and stomach was opened to calculate the ulcer index by Kunchandy method⁶.

Statistical Analysis: The treated group is compared with control and standard group; results were expressed as a mean \pm SD of four animals in each group. The results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P \leq 0.05$ when compared with control is considered significant. Probability table is used to interpret the results.

RESULTS

Preliminary Phytochemical Screening:

The MENS was subjected to chemical tests as per the standard methods for the identification of the various constituents. The results of phytochemical analysis were given in table: 1

Acute toxicity studies

Male albino rats weighing between 150-200 g were selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD- 423 guidelines (acute class method). The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose.

If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/ kg body weight. The animals were observed for toxic symptoms

Table 1 Qualitative Phytochemical Screening

S. No	Phytoconstituents	Methanol Extract (Soxhalation)
1.	Carbohydrates & Glycosides	+
2.	Alkaloids	-
3.	Saponins	-
4.	Flavonoids	+
5.	Proteins & Amino acids	-

Table 2: Effect of alcoholic extract of *Nigella sativa* Linn. on gastric ulcer induced by pylorus ligation in rats.

Design of treatment	Dose	Volume of gastric secretion (ml/100g)	Free acid (mEq/l)	Total acid (mEq/l)	Ulcer score
Group I – Control (normal saline)	2 ml/kg	8.5 ± 0.22	25.6 ± 0.04	60 ± 0.30	2.8 ± 0.07
GroupII - Ranitidine	20mg/kg	4.1 ± 0.01	9.1 ± 0.02	20.3 ± 0.19	1.0 ± 0.08
GroupIII - Alcoholic extract of <i>Nigella sativa</i>	150 mg/kg	5.1 ± 0.01 *	10.4 ± 0.03 *	30.6 ± 0.01 *	1.4 ± 0.01 *

Values are mean ± SEM (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01 vs ulcer control using one way ANOVA followed by Dunnet's test.

Table 3: Effect of alcoholic extract of *Nigella sativa* Linn. on aspirin-induced gastric ulcer in rats.

Group	Design of treatment	Dose	Ulcer score	Percentage protection from ulcer
Group I	Control (normal saline)	2 ml/kg	3.2 ± 0.33	---
Group II	Ranitidine	20 mg/kg	1.0 ± 0.01	68.75
Group III	Alcoholic extract	150 mg/kg	1.6 ± 0.012 *	50.00

Values are mean ± SEM (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01 vs ulcer control using one way ANOVA followed by Dunnet's test.

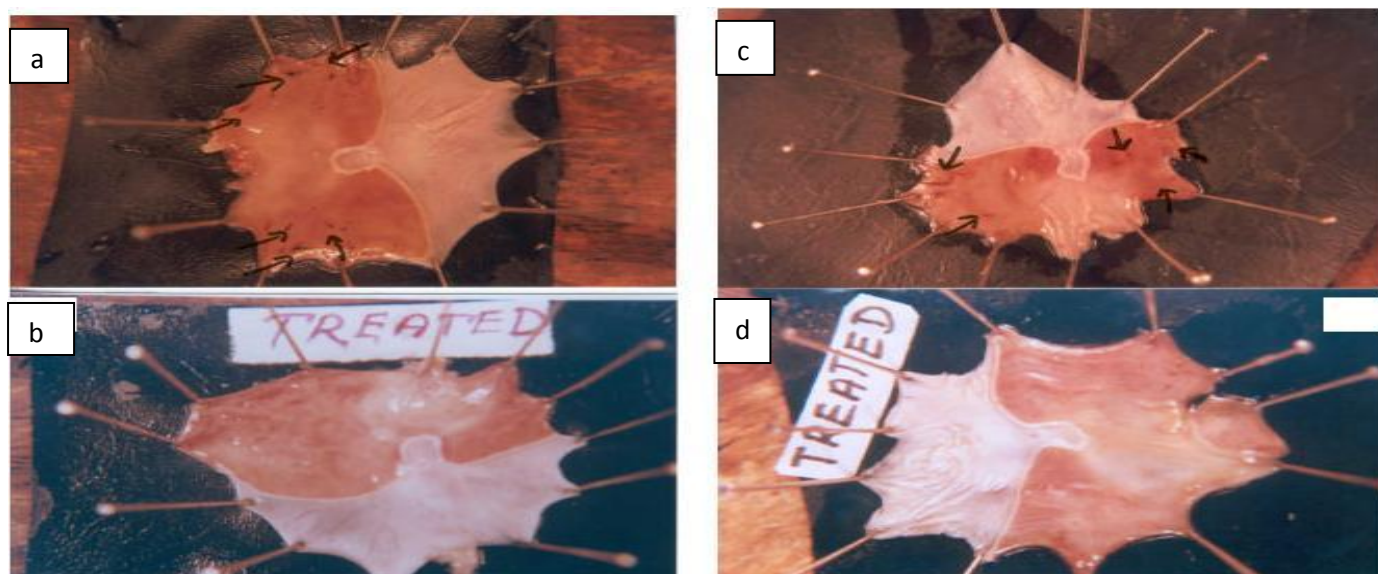


Figure 1. Stomach of **a**, control animal – pylorus ligated ulcer in rat; **b**, test drug treated animal – pylorus ligated ulcer in rat; **c**, control animal – aspirin-induced gastric ulcer in rat; **d**, test drug treated animal – aspirin-induced gastric ulcer in rat.

such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

DISCUSSION

The effect of alcoholic extract of *N. sativa* on pylorus ligated rat and aspirin-induced ulcer models is presented in Tables 2 and 3, respectively. The results of the present study indicate that the alcoholic extract significantly reduces the total volume of gastric juice, free and total acidity of gastric secretion (Tables 2) and also has activity against gastric ulcers in rats.

The control animals had ulcers and haemorrhagic streaks; whereas in animals administered with the extract of *N. sativa* there was significant reduction in ulcer index ($P < 0.005$) (Figure 1). It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells. The preliminary phytochemical studies revealed the presence of flavonoids in alcoholic extract of *N. sativa*; various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. So the possible mechanism of anti-ulcer action of *N. sativa* may be due to its flavonoid content. In this study we observed that *N. Sativa* Provides Significant Anti-Ulcer Activity against Gastric Ulcers in Rats.

CONCLUSION

From our present study it is concluded that Methanol extract of *Nigella sativa* has got moderate anti-ulcer potential against pylorus ligated rat and aspirin-induced ulcer models. The preliminary phytochemical studies revealed the presence of flavonoids in alcoholic extract of *N. sativa*; various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. So the possible mechanism of anti-ulcer action of *N. sativa* may be due to its flavonoid content. In this study we observed that *N. sativa* provides significant anti-ulcer activity against gastric ulcers in rats. However no clear inference can be drawn at this stage

and hence we consider the work for further extensive research.

Acknowledgement

The authors acknowledge the assistance of Dr. Roshan. S (Principal, AZAD college of Pharmacy, moinabad) for his help in carrying out the experiments.

REFERENCES

1. Alberto J, Nunez-Selles- Antioxidant therapy: Myth or reality? Journal of Brazil Chemistry Society 2005, V01 16, No 4, 699-710.
2. Andrade-Cetto Adolfo, Heinrich Michael- Mexican plants with hypoglycaemic effects used in the treatment of diabetes. Journal of Ethnopharmacology 99 (2005). 325-348
3. Ane Waugh and Allison grant 2006-Ross and Wilson. Anatomy and Physiology in health and illness. 10th edition, Churchill livingstone Elsevier Ltd London, 232-234
4. Ansari MA El, Reddy KK, Sastry KNS, Nayudarnma Y- Dicotyledonae Anacardiaceae, Polyphenols of *Mangzfera indica*, Phytochemistry 1971, V01 10, 2239-2241
5. Arya Vaidya, Indian medicinal plants, V01 3, Orientom Longon Publication, 380-383
6. Atta-Ur-Rhemann, Khurshid Zaman, 1989. Medicinal plants with hypoglycemic activity. Journal of Ethnopharmacology 26, 1-55.
7. Balu S, Alagesaboopathi C, Madhavan S. Botanical remedies for diabetes from the canvery delta of Tamilnadu. In: J.K. Maheshwari Editor ethnobotani and medicinal plants of Indian subcontinent, scientific publisher India Jodhpur 1999; 359-62.
8. Badole S, Patel N, Bodhankar S, Jain B, Bhardwaj S- Antihyperglycemic activity of aqueous extracts of leaves of *Cocculus hirsutus* (L) Diels in alloxan induced diabetic mice. Indian journal of Pharmacology. 2006, Vo1 38, issue 1, 49-53
9. Briones, E.R., Mao, S.J.T., Palumbo, P.J., O'Fallon, W.M., Chenoweth, W., Kottke, B.A., 1984. Analysis of plasma lipids and lipoproteins in insulin dependent and non-insulin dependent diabetics. Metabolism 33, 42-49.